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A New Genus and a New Species of Cladorchiidae (Digenea: Dadayiinae) from *Podocnemis expansa* (Chelonia) of the Neotropical Region, State of Pará, Brazil

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A NEW GENUS AND A NEW SPECIES OF CLADORCHIIDAE (DIGENEA: DADAYIINAE) FROM *PODOCNEMIS EXPANSA* (CHELONIA) OF THE NEOTROPICAL REGION, STATE OF PARÁ, BRAZIL

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ABSTRACT: A new species of amphistome digenean from the stomach and intestine of *Podocnemis expansa* (Pelomedusidae), of the tropical rain forest, from the State of Pará, Brazil, is described and allocated to a new genus (*Oriximinatrema noronhae*). The new species is characterized by the presence of an esophageal bulb, an esophageal extension uncovered by an extension of the pharyngeal sacs, a well-developed cirrus sac, post-bifurcal genital sucker, a ventro-terminal acetabulum with an anterior lip, and medium-sized eggs. This is the first report of a Dadayiinae trematode infecting a reptilian host.

The geographic distribution of *Podocnemis expansa* Schweigger, 1812 (Reptilia: Chelonia: Podoneminae) (the giant South American river turtle) includes the Caribbean drainages of Guyana and Venezuela, and the upper Amazon tributaries in Bolivia, Peru, Colombia, Venezuela, and Brazil. The species is also occasionally found in Trinidad, especially after Orinoco River flooding (Ernst and Barbour, 1989).

Species of *Podocnemis* are hosts for 4 species of amphistome digeneans, namely, *Nematophila grandis* (Diesing, 1836) Travassos, 1934, *Nematophila venezuelensis* (Cordero & Vogelsang, 1940) Yamaguti, 1958, *Halltrema heteroxenus* (Cordero & Vogelsang, 1940) Jones, 2005, and *Halltrema avitellinum* Lent & Freitas, 1939 (Travassos, 1934; Lent and Freitas, 1939; Yamaguti, 1971; Sey, 1991; Jones, 2005b).

Herein, we describe a new species of amphistome parasitizing specimens of *P. expansa* from the State of Pará, Brazil, and we propose a new genus for it.

MATERIALS AND METHODS

Helminths collected from 33 parasitized host specimens out of 50 *Podocnemis expansa* individuals from Oriximiná, State of Pará, Brazil, were received by the Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (LHPV, IOC, FIOCRUZ). Four of the 33 host specimens were parasitized with a total of 20 specimens of the digenean described herein. Parasites were fixed in AFA, uncompressed, stained in Delafield's hematoxylin, dehydrated in an ethanol series, cleared in beechwood creosote, and mounted in Canada balsam in accordance with procedures outlined by Eiras et al. (2006). Procedures to obtain histological sections and terminology followed Sey (1991), and the taxonomic classification follows Jones (2005a, 2005b). Measurements are in millimeters (mm); the range is followed by means in parentheses, together with the number of measured specimens. The total number of parasitized specimens and the infrapopulation of each host are indicated in the taxonomic summaries. The prevalence and the intensity of infection are indicated as follows: number of hosts positive for trematodes/number hosts examined: size of each infrapopulation. The use of ecological terms is based on Bush et al. (1997). Holotype and paratypes were deposited in the Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil.

For comparative purposes, samples of *Curimatrema microscopica* were also obtained from the Coleção de Invertebrados Não-Insecta do Instituto Nacional de Pesquisas da Amazônia (CI-INPA). Drawings were made with the aid of a drawing tube connected to a bright-field Olympus BX41 microscope. Photographs were taken with a digital camera using a Zeiss Axiophot bright-field microscope, with and without differential interference contrast (DIC) apparatus, and confocal laser scanning micrographs were taken using a Zeiss LSM 510 microscope (CLSM). The authorization for the capture of the hosts and collection of parasites was obtained by the Centro Nacional de Quelônios Aquáticos (CENAQUA) from the Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis (IBAMA) no. 085.

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DESCRIPTION

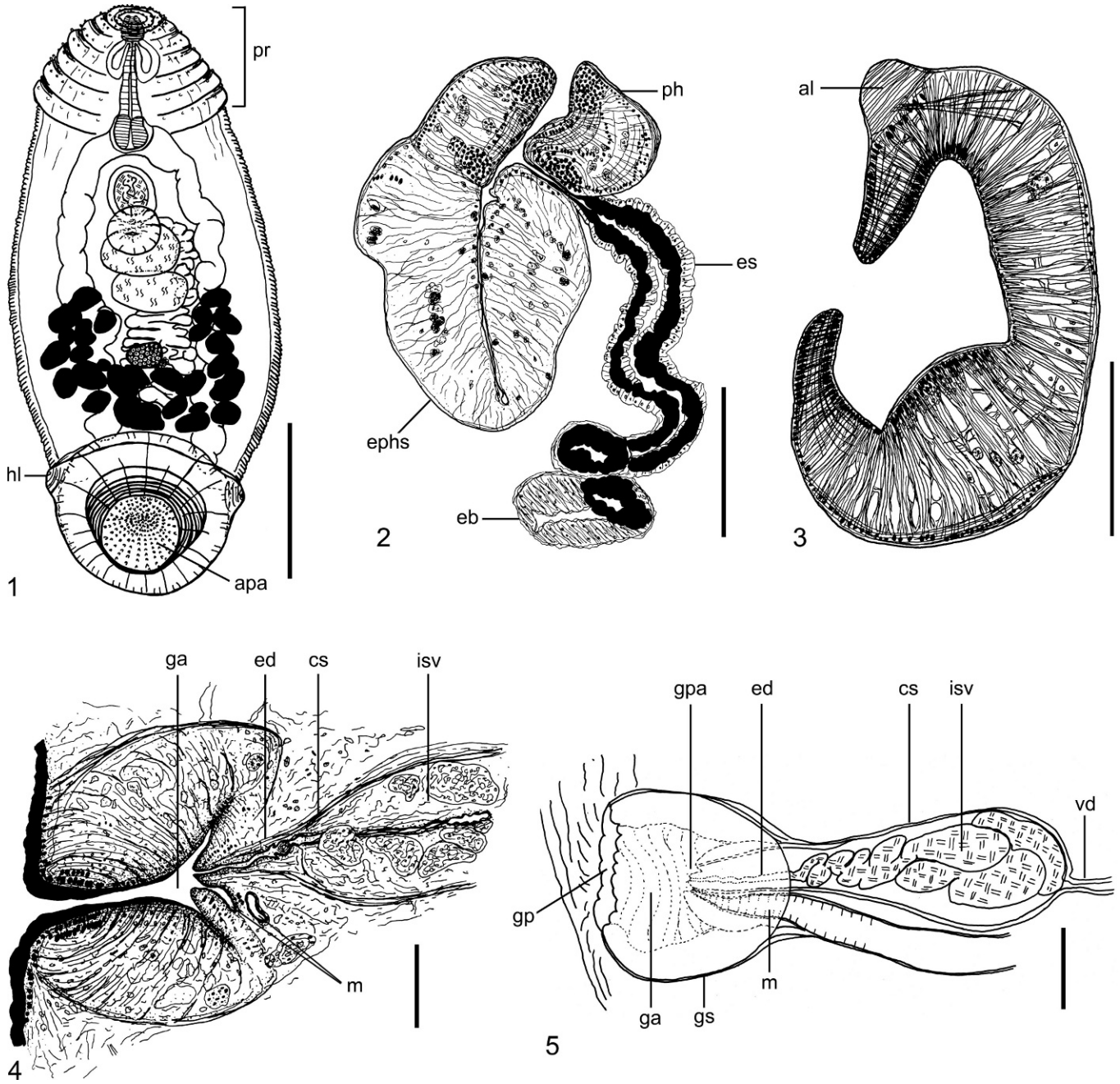
Oriximinatrema n. gen.

Diagnosis: Cladorchiidae Fischoeder, 1901, Dadayiinae Fukui, 1929. Body thin, slightly oval, and elongated, with a ridge encircling anterior extremity. Tegumentary papillae present on ridges encircling anterior extremity. Pharynx well developed. Prominent primary extramural pharyngeal sacs. Esophagus with muscular bulb. Ceca undulating, terminating at anterior margin of acetabulum. Acetabulum ventro-terminal, with anterior overhanging lip containing pair of lateral horn-like projections. Testes tandem, margins smooth, wider than long, almost always juxtaposed, in middle third of body. Cirrus sac oval, well developed, post-bifurcal, containing seminal vesicle. Ovary elliptical, entire, median, compact, intercecal, post-equatorial, separated from acetabulum and posterior testis. Mehlis' gland post-ovarian. Uterus intercecal, extending to posterior portion of body, close to anterior rim of acetabulum, and extending to anterior portion toward atrium, passing dorso-laterally to testes. Laurer's canal inconspicuous. Metraterm present. Genital sucker present, post-bifurcal. Eggs medium sized. Vitelline follicles large, extending in extra-, infra-, supracecal fields from level of posterior testes to just before cecal termination, confluent in post-ovarian area. Excretory pore, median, on dorsal surface, posterior to end of cecal tips. Excretory vesicle I-shaped. Lymphatic system with single pair of longitudinal trunks.

Oriximinatrema noronhae n. sp.

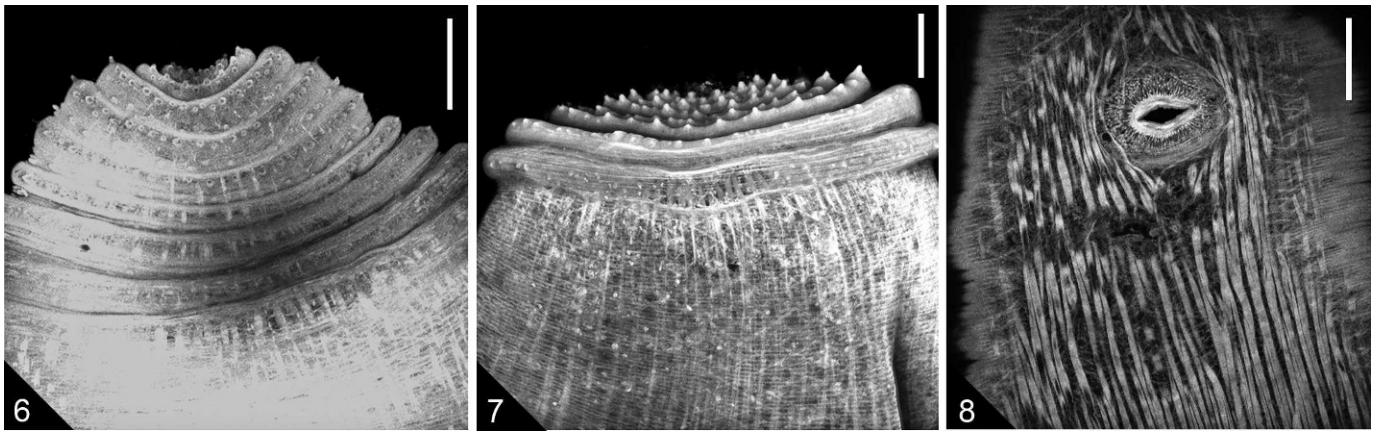
(Figs. 1–8)

Diagnosis (based on 12 adult specimens 8 whole mounts, and 4 vertical-longitudinally sectioned): Body 1.10–2.75 (1.69, n = 8) long by 0.48–1.05 (0.67, n = 8) wide, tiny, slightly elongated oval with ridge encircling anterior extremity. From mouth to bulbar region, tegument with 8 rows of parallel, transverse, prominent, tegumentary, papillated ridges or hoops (Fig. 1). Bands of longitudinal, radial, and circular muscles present (Figs. 6, 7). Oral opening terminal surrounded with papillae (Figs. 1, 6). Pharynx 0.13–0.17 (0.15, n = 2) in length; maximum width 0.16–0.18 (0.17, n = 2); well developed, with a set of muscular units: exterior, interior, and middle muscle circular units moderately well developed; longitudinal fibers, exterior fibers more developed than interior; and radial fibers weakly developed. Pharynx with anterior and posterior sphincter (Fig. 2). Prominent primary extramural pharyngeal sacs 0.10–0.23 (0.16, n = 4) long, minimum width 0.03 (n = 2), maximum width 0.14–0.18 (0.16, n = 3) (Fig. 2). Esophagus with muscular bulb. Esophagus excluding bulb 0.10–0.20 (0.15, n = 2) long and 0.01(n = 2) wide. Esophageal bulb 0.12–0.18 (0.14, n = 3) long; maximum width 0.12–0.14 (0.13, n = 3) (Fig. 2). Ceca running laterally at first and then turning posteriorly, undulating;



FIGURES 1–5. *Oriximinatrema noronhae* n. gen., n. sp. (**1**) Entire worm, ventral view. (**2**) Pharynx in median sagittal section. (**3**) Acetabulum in median sagittal section. (**4**) Terminal genitalia in median sagittal section. (**5**) Terminal genitalia lateral view. (al = acetabulum overhanging lip; apa = acetabular papillae; cs = cirrus sac; eb = esophageal bulb; ed = ejaculatory duct; ephs = extramural pharyngeal sac; es = esophagus; ga = genital atrium; gp = genital pore; gs = genital sucker; hl = lateral horn-like projection; isv = internal seminal vesicle; m = metraterm; ph = pharynx; pr = papillated ridges; vd = vas deferens). Scale bars: Fig. 1 = 0.25 mm; Fig. 2 = 0.04 mm; Fig. 3 = 0.1 mm; Fig. 4 = 0.02 mm; Fig. 5 = 0.05 mm.

terminating at anterior margin of acetabulum in ventral view, non-confluent (Fig. 1). Right cecum 0.70–1.40 (0.98, n = 7) long, terminating 0.32–0.51 (0.42, n = 7) from posterior margin of acetabulum. Left cecum 0.76–1.40 (0.99, n = 7) long, terminating at 0.30–0.56 (0.41, n = 7) from posterior margin of acetabulum. Acetabulum 0.29–0.64 (0.39, n = 7) long by 0.38–0.76 (0.50, n = 5) wide, ventro-terminal, with anterior overhanging lip containing pair of lateral horn-like projections (Fig. 1). Inner surface covered with papillae (Fig. 1). Acetabulum musculature, wall and cavity well developed. Radial fibers well developed, forming smaller bundles. Exterior longitudinal series well developed. Dorsal exterior circular (dec; Sey, 1991), units present, well developed (around 30 units), divided into dec_1 (around 20 units), outer, and dec_2 (around 10 units), inner. Dorsal interior circular units present, well developed (around 35 units). Dorsal interior units in larger number end more developed than dorsal exterior units. Ventral interior circular units (around 50 units) and exterior circular units (around 80 units) present. Ventral exterior units in larger number than interior units. Ventral interior units well developed, growing smaller and then larger toward median section. Ventral exterior units well developed, growing smaller toward median section. Median circular interior and exterior units moderately developed. Median interior units (around 30 units) larger in number than median exterior units (around 10 units). Oblique fibers present dorsally and ventrally. Horn-like projection with



FIGURES 6–8. *Oriximinatrema noronhae* n. gen., n. sp., micrographs by LCSM. (**6**) Anterior extremity, relaxed specimen. (**7**) Anterior extremity, contracted specimen. (**8**) Genital sucker. Scale bars Figures 6 and 8 = 100 μm; Figure 7 = 50 μm.

well-developed oblique fibers above dec_2. Internal circular units fewer than external circular series (Fig. 3).

Testes tandem, entire, wider than long, almost always juxtaposed, in middle third of body (Fig. 1). Anterior testis 0.06–0.19 (0.11, n = 7) long by 0.19–0.34 (0.24, n = 7) wide; posterior testis 0.06–0.17 (0.10, n = 7) long by 0.17–0.37 (0.27, n = 7) wide. Seminal vesicle 0.15 long by 0.13 wide (n = 1), oval, enclosed in cirrus sac. Cirrus sac 0.21 long by 0.13 wide (n = 1), oval, well developed, with poorly developed musculature, post-bifurcal, median, anterior to testes, overlapped by anterior portion of genital sucker in ventral view (Fig. 4). Ejaculatory duct opening to atrium anteriorly, separately from metraterm. Vas deferens entering cirrus sac dorsally (Fig. 5). Ovary 0.05–0.1 (0.08, n = 4) long by 0.08–0.17 (0.12, n = 4) wide, elliptical, entire, median, compact, intercecal, post-equatorial, post-testicular, separated by uterine coils from posterior testis, closer to posterior testes than acetabulum (Fig. 1). Mehlis' gland, sinistral, posterior to ovary. Uterus intercecal, some coils post-ovarian, extending close to the anterior rim of the acetabulum, extending to anterior portion toward atrium passing dorso-laterally to testes (Fig. 1). Laurer's canal inconspicuous. Metraterm opening in atrium posterior to ejaculatory duct (Figs. 4, 5). Genital sucker 0.09–0.20 (0.14, n = 5) long by 0.12–0.22 (0.17, n = 4) wide, with prominent sphincter, ventral, median, post-bifurcal, overlapping anterior testes at anterior border (Figs. 1, 4, 5, 8). Genital papillae short, stocky with sphincter papillae (Figs. 4, 5). Eggs 0.06–0.11 (0.08, n = 10) long by 0.05–0.07 (0.06, n = 10) wide. Vitelline follicles large, extending in extra-, infra-, and supracecal fields from level of posterior testes to immediately posterior to cecal termination, confluent in post-ovarian area (Fig. 1). Excretory pore, median, on dorsal surface, posterior to cecal tips. Excretory vesicle I-shaped. Lymphatic system with 1 pair of longitudinal trunks.

Taxonomic summary

Type host: Podocnemis expansa Schweigger, 1812, Chelonia, Pelomedusidae; giant South American river turtle.

Type locality: Oriximiná (01°45′55″S, 55°51′50″W), State of Pará, Brazil.

Sites of infection: Stomach and intestine.

Prevalence and intensity of infection: Four hosts of 50 examined; 1, 15, 1, and 3.

Material deposited: CHIOC no. 36633a holotype (whole mount) and 36633b, 36634a–h, 36635 paratypes (whole mounts).

Etymology: The generic name derives from the type locality, and the specific name is in honor of the recently retired CHIOC curator Dr. Dely Noronha.

Material examined: Curimatrema microscopica CI-INPA no. 373 holotype and 374a–g paratypes.

Remarks

The new species belongs in the Cladorchiidae (sensu Jones, 2005a); diagnostic features include the presence of a sucker-like attachment organ, 2 testes, normal gut structure, paired extramural pharyngeal sacs, cirrus sac and a male duct without a massive dilated chamber, and no pharyngeal bulb. The vitelline follicles extend from the level of the posterior testes to just before the cecal termination; *Oriximinatrema* belongs within the Dadayiinae.

In this subfamily, the most similar genus is *Curimatrema* Thatcher, 2000, with anterior end of body demarcated by collar-like circular ridge, pharynx with extramural sacs and esophagus with a bulb, genital sucker, subspherical testes, and submedian ovary (Thatcher, 2000; Jones, 2005a). The new genus, however, differs from *Curimatrema* in a number of ways: (1) The pharyngeal extramural sacs extend to the middle region of the esophagus; (2) the testes are tandem; (3) the genital sucker is post-bifurcal; (4) the acetabulum is ventro-terminal, with an anterior overhanging lip having a pair of lateral small horn-like projections; and (4) the eggs are medium sized.

DISCUSSION

Following the taxonomic key to the Cladorchiidae provided by Jones (2005b), the new genus was allocated to Dadayiinae or Schizamphistominae, based on (1) presence of a non-intertesticular excretory vesicle, (2) intercecal testes, (3) non-extensive extracecal loops in the uterus, (4) non-bipartite extramural pharyngeal sacs, and (5) vitelline follicles extending to the testicular zone. No members of the Schizamphistominae possess genital suckers similar to that observed in the specimens described herein. Three members of the Dadayiinae, however, posses genital suckers: *Pacudistoma*, *Curimatrema*, and *Dadayius.* Species of *Pacudistoma* differ from the new species in possessing a massive pharynx with intramural sacs, an esophagus without a bulb, a genital pore opening immediately post-bifurcal, lobate testes, and small vitelline follicles, ventral and internal to the ceca. Species of *Curimatrema* have a subterminal acetabulum, diagonal subspherical testes, a bifurcal genital pore, and large eggs. Species of *Dadayius* have a massive acetabulum, transverse ridges on the terminal surface, rounded or oval testes, a bifurcal or prebifurcal genital pore, and no cirrus sac (Jones, 2005b). In contrast, specimens of the new species have terminal acetabulum, testes tandem, a post-bifurcal genital pore, and medium-sized eggs. On the basis of these differences, *Oriximinatrema* n. gen. is proposed within the Dadayiinae. This is the first report of this subfamily in a reptilian host, the South American river turtle; all other members have been found in freshwater fishes.

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